

Marine Bioluminescence: Mechanisms and Evaluation

James F. Case
Marine Science Institute
University of California Santa Barbara
Santa Barbara, CA 93106
Phone: (805) 893-3639 fax: (805) 893-8062 email: case@lifesci.ucsb.edu

Award #: N00014-97-1-0424

LONG TERM GOALS

Our long-term goal is still to understand the mechanisms and adaptive significance of marine bioluminescence (BL). The ubiquity and immense variety of marine BL argues that marine bioluminescence most probably has major unknown significance to life in the sea (Case, et al., 1995).

OBJECTIVES

In this report we emphasize our efforts towards elucidating the intracellular processes involved in sensing a mechanical stimulus and transducing that stimulus into a biochemical reaction resulting in light output. Bioluminescent dinoflagellates are ideal for such work because they are free-living single-celled organisms, making it relatively simple to examine intracellular transduction events; and they are commonly the major producers of surface bioluminescence in the ocean.

Bioluminescence in dinoflagellates has been shown to be maximally triggered by hydrodynamic laminar shear forces (Latz, Case and Gran 1994). These shear forces stimulate a mechanosensor that somehow triggers an action potential (AP) along the inner, vacuolar membrane (Widder and Case 1981). This AP is thought to cause the release of H⁺ from the acidic vacuole into the cytoplasm. The luciferin-luciferase reaction in dinoflagellates is known to be triggered by a lowering of the pH of the surrounding medium to pH 6 (Hastings and Dunlap 1986). This entire process takes approximately 20 ms, which is faster than similar mammalian mechanotransduction systems.

Our efforts are involved in elucidating the mechanism by which the mechanosensor is linked to the vacuolar AP, which eventually triggers the BL reaction and how a circadian process may modify this linkage. In the past, much of the research characterizing the signal transduction in bioluminescent dinoflagellates has been performed on two species, *Lingulodinium polyedrum* and *Pyrocystis fusiformis*. We are continuing to study these two organisms, focusing on *P. fusiformis*.

Currently, we are specifically concentrating on the role of the cytoskeleton in sensing mechanical forces. The cytoskeleton has been shown to provide a cell structural integrity, responding to external mechanical stimuli through redistribution and changes in concentration. Studies with mammalian cells have indicated that the cytoskeleton is an integral component of the mechanotransduction machinery. Therefore, the role of the cytoskeleton in mechanotransduction in *P. fusiformis* is being examined.

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 30 SEP 2001		2. REPORT TYPE		3. DATES COVERED 00-00-2001 to 00-00-2001	
4. TITLE AND SUBTITLE Marine Bioluminescence: Mechanisms and Evaluation				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Marine Science Institute, University of California Santa Barbara,, Santa Barbara,, CA, 93106				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 5	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

APPROACH

Past experiments in this laboratory have characterized the distribution of the filamentous actin (f-actin) portion of the cytoskeleton as well as its sensitivity to high doses of cytochalasin-D, a known f-actin depolymerizing drug. To further characterize the sensitivity of the f-actin cytoskeleton to cytochalasin-D (CD), dose response studies have been performed. The presence and amount of f-actin is being quantified following treatment from various CD concentrations. This characterization of the effectiveness of CD treatment is necessary in that CD in different concentrations can be used to modify and/or destroy the f-actin cytoskeleton. However the effective concentrations for minor modification versus total disruption vary from species to species.

Following preliminary experiments using an integrating sphere and PMT system to record BL output of cells treated with CD and exposed to low-level shear, it was found that CD has a significant effect on the mechanical stimulability of the BL. However, the system was imperfect and a modification of the stimulation mechanism was necessary to more fully characterize and control the type of stimulation being delivered to the cells. The past year has included an effort to retool the sphere's stimulation delivery method to create a more precise and quantifiable shear field to which cells are exposed and their BL output recorded.

After the sphere system was improved, work was resumed that involved characterizing the role of the f-actin cytoskeleton in transducing fluid shear to trigger the BL reaction. The f-actin cytoskeleton was modified or totally depolymerized (using different concentrations of CD) and the ability to mechanically stimulate BL was measured. *P. fusiformis* cells were exposed to this drug and then their ability to produce BL in response to fluid shear was compared with control cells. A dose-response curve is being created based on the sensitivity changes of the BL system at the different concentrations of CD tested. Also being tested is the cells' BL response to different levels of shear following either no treatment (control) or treatment with a predetermined effective concentration of CD.

WORK COMPLETED

The shear stimulation mechanism inside of the integrating sphere was modified and improved so that it delivers more precise, controlled and repeatable laminar shear.

We are continuing to characterize the role of the f-actin cytoskeleton in sensing laminar fluid shear as expressed by the BL system.

We are in the process of determining the dose-response characteristics of the *P. fusiformis* f-actin cytoskeleton to cytochalasin-D.

We have further quantified and described the circadian movement of the chloroplasts in *P. fusiformis* along the f-actin cytoskeleton.

RESULTS

We found that mechanically stimulated BL output in *P. fusiformis* was increased upon depolymerizing the filamentous actin cytoskeleton. This finding indicates that the filamentous actin cytoskeleton is not a necessary component in BL mechanotransduction in *P. fusiformis*, but does appear to modulate the sensitivity to mechanical forces. It is hypothesized that the filamentous actin cytoskeleton is involved

with providing the cell with the structural integrity necessary to resist mechanical forces, thus, decreasing the amount of filamentous actin resulted in increased sensitivity and increased BL response.

We have quantified the rate of chloroplast migration that occurs on a circadian rhythm. The migration rates were highly variable with some clusters of chloroplasts moving at rates in excess of 100 nm/s whereas some individual chloroplasts were found to move at 50 nm/s. Both of these rates match intracellular movement rates powered by certain classes of f-actin motors (myosins), as determined by other research groups.

IMPACT/APPLICATIONS

The dinoflagellate cytoskeleton is a dynamic cellular component that has the ability to respond to environmental stimuli quickly and controls a multitude of intracellular processes ranging from triggering BL to organelle migration.

No one has ever before examined the role of the cytoskeleton in the BL response. Our findings indicate that the state and concentration of the filamentous actin cytoskeleton dramatically affects the amount of BL triggered by fluid shear. Thus, it would appear that the filamentous actin cytoskeleton is involved in regulating the sensitivity to mechanical forces and may be able to change the threshold for stimulating BL in dinoflagellates. However, the filamentous actin cytoskeleton does not seem to be a necessary component of the mechanotransduction machinery, but rather functions as a modulating element to the sensitivity of mechanical forces.

It has long been argued that the actin cytoskeleton is the main intracellular structure involved in sensing mechanical forces. This has recently been contested with evidence indicating that the cytoskeleton is not necessary for mechanoreception to occur. Since bioluminescent dinoflagellates are such exquisitely sensitive shear sensors and their sensitivity is easily measured, they are ideal study organisms for this type of question.

No metazoan bioluminescence system has ever been completely characterized from stimulus to bioluminescent output, particularly the steps involved in the transduction of the stimulus energy to the bioluminescence reaction. The mechanisms found to control dinoflagellate bioluminescence could provide a model for studying bioluminescence control mechanisms in other organisms.

REFERENCES

Case, J.F., S.H.D. Haddock and R. D. Harper (1995) The ecology of bioluminescence. Proc. 9th Int. Symposium on Bioluminescence and Chemiluminescence.

Latz, MI, JF Case and RL Gran (1994) Excitation of bioluminescence by laminar fluid shear associated with simple couette flow. *Limnol. Oceanogr.* 39(6):1424-1439.

Widder, EA and JF Case (1981) Bioluminescence excitation in a dinoflagellate, p. 125-132, in KH Neilson, ed. *Bioluminescence current perspectives*. Burgess.

PUBLICATIONS

McDougall, CA, C. Kuzminski, JF Case (2000) Mechanoscentisity in a bioluminescent dinoflagellate:role of the actin cytoskeleton.. IX International Symposium on Bioluminescence. p 64.

Mallefet, J, CA McDougall, CM Herren, G. Hendler and JF Case (2000) Observations on a new luminous ophiuroid (Echinodermata) IX International Symposium on Bioluminescence. p. 64